AD-A193 263 MUTAGENIC POTENTIAL OF NITROSOGUANIDINE IN THE AMES SALMONELLA/MAMMALIAN..(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA.. 1/1

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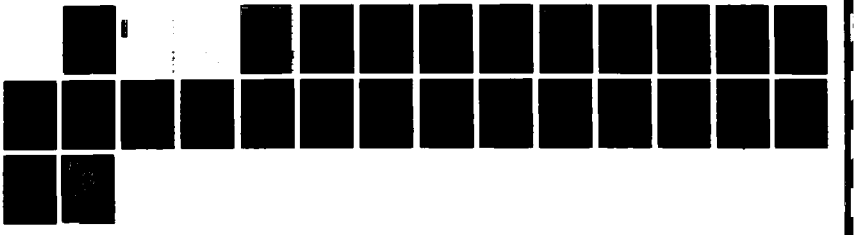

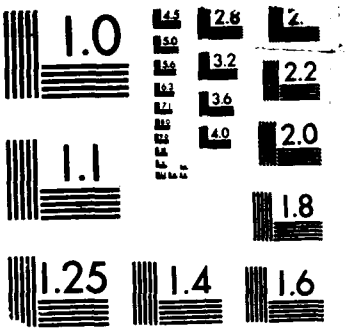

MICROCOPY RESOLUTION TEST CHART
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Mutagenic potential of nitrosoguanidine in the Ames Salmonella/Mammalian Microsome Mutagenicity Test

12. PERSONAL AUTHOR(S): Suzanne E. Sebastian and Don W. Korte, Jr.

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FIELD	GROUP	SUB-GROUP	mutagenicity, genetic toxicology, Ames test, NITROSOGUANIDINE

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The mutagenic potential of NITROSOGUANIDINE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.05 mg/plate to 0.0875 mg/plate. The test compound was not mutagenic under conditions of this test.

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ABSTRACT

The mutagenic potential of NITROSOGUANIDINE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.05 mg/plate to 0.0875 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, NITROSOGUANIDINE.

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5010
Project Officer: Gunda Reddy, PhD.

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLBO

GLP STUDY NUMBER: 86008

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: Suzanne E. Sebastian, BA, SP4, USA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: NITROSOGUANIDINE

INCLUSIVE STUDY DATES: 28-30 October, 1986

OBJECTIVE: The objective of this study was to determine the mutagenic potential of NITROSOGUANIDINE (LAIR Code TP 48) by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC; SGT Lillie D. Witcher, BS; and SGT Gayle A. Orner, BS, provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 86008 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 16 FEB 88

DON W. KORTE, Jr., PhD / Date
MAJ, MSC
Study Director

Suzanne E. Sebastian 12 February 88

SUZANNE E. SEBASTIAN, BA / DATE
SP4, USA
Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA (70-1n) 23 February 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for 86008, Tox Series 148

1. I hereby certify that the protocol was reviewed on 29 October 1986.

2. The report and raw data for this study were audited on 1 December 1987.

Carolyn M. Lewis
CAROLYN M. LEWIS
C, Quality Assurance

TABLE OF CONTENTS

Mutagenic Potential of NITROSOGUANIDINE - Sebastian and Korte

Nitrosoguanidine is an anticipated environmental degradative product of nitroguanidine (1). Nitroguanidine, a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its charge to evaluate the environmental and health hazards of propellants generated by US Army munitions manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (2). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine and related intermediates/by-products of its manufacture or environmental degradation products.

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of Salmonella typhimurium to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating in vivo metabolic activation of the test compound. The Ames test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (3).

This evaluation of NITROSOGUANIDINE utilizes a revision of the Ames Salmonella/ Mammalian Microsome Mutagenicity Test (4). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set.

Objective of the Study

The objective of this study was to determine the mutagenic potential of NITROSOGUANIDINE (LAIR Code TP 48) by using the revised Ames Salmonella/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical name: NITROSOGUANIDINE

Code number: LAIR Code No. TP 48

Physical state: White crystalline solid

Source: Alan Rosencrance
US Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5010

Storage: NITROSOGUANIDINE was received from USABRDL and was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Nitrosoguanidine was characterized for chemical composition and purity by the Division of Toxicology, LAIR (Presidio of San Francisco, CA). The purity of nitrosoguanidine was 97.5% with about 2.5% nitroguanidine. Results of this analysis are presented in Appendix A.

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test chemical was dissolved in the same lot of DMSO. Reagent grade water used in this assay was first passed through a Technic Series 300 Reverse Osmosis Unit (Seattle, WA), then through a Corning MP-1 Mega Pure System glass distillation unit (Corning Glass Works, Corning, NY) (5).

Chemical Preparation

On the day of testing, the compound was dissolved directly into DMSO at a concentration of 15 mg/ml. Aliquots of this solution were used to prepare the serial dilutions.

Test Strains

Salmonella strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 obtained from the laboratory of Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (6).

Mammalian Microsome System

The S-9 (batch #R-315) was purchased from Microbiological Associates Inc. (Bethesda, MD). The optimal titer of this S-9, as determined by Microbiological Associates Inc., was 0.75 mg protein/plate.

Test Format

NITROSOGUANIDINE was evaluated for mutagenic potential according to the revised Ames method (4). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (6). The preincubation modification was chosen to enhance the sensitivity of the assay by exposing the bacteria to higher concentrations of test compound (and the activation products, when present) than was possible in the standard plate incorporation assay. The bacteria were preincubated in the presence of the compound, both with and without metabolic activation, for 20 minutes on a shaker incubator at 37°C. A single preincubation tube was prepared for each top agar triplicate. Each preincubation tube contained 10 ml of a mixture which consisted of 1 ml of bacteria (16 hour culture), 1 ml of test compound (15 mg/ml in DMSO or a serial dilution), 2 ml of S-9 mixture if required, and the remaining volume nutrient broth. The highest dose (1.5 mg/ml) in the preincubation mixture approached the practical limits of solubility for nitrosoguanidine in aqueous media (Appendix A). The top agar tubes were prepared by adding 0.7 ml of the preincubation mixture to 2 ml of top agar. After mixing, the top agar was then overlaid on minimal glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (7). Plates were incubated upside down in the dark at 37°C for 72 hours (Maron 1985, personal communication). Plates were prepared in triplicate and the individual revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control).

The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Maron and Ames (4). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The Salmonella strains were verified by a standard battery of tests. The integrity of the different Salmonella strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicated that the prerequisite alteration of the lipopolysaccharide layer of the cell wall was present.

-Growth in the presence of ampicillin-impregnated disks which indicated the presence of an ampicillin-resistant R Factor in all strains except TA1535, TA1537, and TA1538.

-Lack of growth (inhibition) following exposure to ultraviolet light which indicated the absence of the DNA excision-repair mechanism (for all strains except TA102).

Six known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene (lot 18C-0378), 2-aminofluorene (lot 021547), 2-aminoanthracene (lot 020797), mitomycin-C (lot 015F-0655), 4-nitroquinoline-n-oxide (lot 89C-0710) and N-methyl-N'-nitro-N-nitrosoguanidine (lot 127C-0342), were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (8), a compound is considered

mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538 (4,6). A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (4) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations from the Protocol/SOP

The preincubation modification of the Ames Assay was chosen to enhance sensitivity by exposing bacteria to a higher concentration of compound, for a longer period of time. Volumes for the preincubation mixture were different from those specified in the SOP because of the limited solubility of nitrosoguanidine. This deviation has no impact on the validity of the study.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 21-24 May, 1986 (Table 1). NITROSOGUANIDINE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 2).

A tabular presentation of raw data is included in Appendix B.

TABLE 1

STRAIN VERIFICATION AND STERILITY TESTING FOR
THE MUTAGENICITY DETERMINATION
ON NITROSOGUANIDINE (TP 48)

GLP STUDY NUMBER 86008

STRAIN VERIFICATION

OBSERVATIONS*

STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA97	NG	G	NG	NG	NG
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA102	NG	G	G	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

* G = Growth, NG = No Growth

TABLE 2

NITROSOGUANIDINE

REVERTANTS/PLATE

MEAN ± 1SD

COMPOUND*	DOSE/PLATE	TA97	TA98	TA100	TA102
WITHOUT S-9					
NEG CONTROL	0.0 mg	88 ±16.1	14 ±3.3	111 ±10.2	173 ±13.2
MITO C	0.5 µg				1327 ±22.5
MNNG	2.0 µg			1460 ±149.1	
NQNO	2.0 µg	252 ±92.2			
TP 48	1.05 mg	82 ±16.4	21 ±4.0	108 ±0.7	168 ±9.3
TP 48	0.8 mg	90 ±6.4	20 ±6.0	110 ±9.6	164 ±11.3
TP 48	0.6 mg	91 ±1.5	13 ±3.5	76 ±7.5	172 ±13.4
TP 48	0.4 mg	78 ±4.4	18 ±6.1	106 ±12.9	173 ±15.4
TP 48	0.2 mg	88 ±7.6	19 ±3.1	102 ±4.4	134 ±21.0
TP 48	0.1 mg	83 ±16.4	14 ±4.2	103 ±7.2	165 ±20.4
WITH S-9					
NEG CONTROL	0.0 mg	110 ±16.6	35 ±10.8	125 ±21.7	282 ±5.4
2-AA	2.0 µg		298 ±73.4	563 ±86.5	
2-AF	2.0 µg	572 ±136.7	1683 ±42.5	706 ±86.5	
BP	2.0 µg	251 ±7.5	125 ±7.5	366 ±35.8	
TP 48	1.05 mg	120 ±14.0	33 ±3.6	103	236 ±18.6
TP 48	0.8 mg	108 ±7.8	35 ±5.0	126 ±5.0	258 ±16.1
TP 48	0.6 mg	113 ±6.1	30 ±8.2	105 ±0.7	240 ±4.6
TP 48	0.4 mg	138 ±9.5	29 ±12.7	100	264 ±16.1
TP 48	0.2 mg	117 ±7.6	21 ±1.5	115 ±6.4	228 ±22.7
TP 48	0.1 mg	105 ±22.3	29 ±1.2	134	234 ±26.1

*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 2, (Continued)

NITROSOGUANIDINE

REVERTANTS/PLATE

MEAN ± 1SD

COMPOUND*	DOSE/PLATE	TA1535	TA1537	TA1538
WITHOUT S-9				
NEG CONTROL	0.0 mg	11 ±3.0	10 ±3.2	18 ±6.4
MNNG	20.0 µg	2224 ±378.2		
TP 48	1.05 mg	18 ±3.8	11 ±3.2	15 ±1.0
TP 48	0.8 mg	15 ±3.2	11 ±2.0	14 ±1.0
TP 48	0.6 mg	14 ±3.6	16 ±9.5	14 ±4.2
TP 48	0.4 mg	15 ±2.6	10 ±6.6	16 ±3.6
TP 48	0.2 mg	19 ±4.5	10 ±2.3	13 ±2.1
TP 48	0.1 mg	15 ±2.5	7 ±1.0	14 ±1.5
WITH S-9				
NEG CONTROL	0.0 mg	11 ±1.7	12 ±4.1	27 ±2.4
2-AA	2.0 µg		318 ±55.2	572 ±231.1
2-AF	2.0 µg			1168 ±173.9
BP	2.0 µg		48 ±5.9	74 ±39.0
TP 48	1.05 mg	11 ±4.4	11 ±5.9	28 ±3.1
TP 48	0.8 mg	16 ±9.0	9 ±1.2	27 ±7.1
TP 48	0.6 mg	18 ±2.1	15 ±3.5	23 ±4.9
TP 48	0.4 mg	10 ±3.2	12 ±3.5	20 ±0.6
TP 48	0.2 mg	16 ±4.0	10 ±1.0	23 ±4.0
TP 48	0.1 mg	10 ±1.4	7 ±3.6	21 ±9.1

*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

DISCUSSION

Certain test criteria must be satisfied before an Ames test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the lipopolysaccharide layer, and deficiency in DNA excision-repair (except TA102). Second, the Salmonella strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, NITROSOGUANIDINE was evaluated in the Ames preincubation test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times (TA97, TA98, TA100, TA102) (3,8) or three times (TA1535, TA1537, TA1538) (4,6) the spontaneous revertant colony count. NITROSOGUANIDINE did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that NITROSOGUANIDINE is not mutagenic when evaluated in the Ames test.

CONCLUSION

NITROSOGUANIDINE, both in the presence and absence of metabolic activation, was evaluated for mutagenic potential in the Ames test, and did not induce a positive mutagenic response under conditions of this study.

REFERENCES

1. Kaplan DL, Cornell JH, Kaplan AM. Decomposition of nitroguanidine. Environ Sci Technol 1982: 16:488-492.

2. Kenyon KF. A data base assessment of environmental fate aspects of nitroguanidine. Frederick, Maryland: US Army Bioengineering Research and Development Laboratory, 1982, DTIC No. ADA 125591.

3. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/Mammalian Microsome Mutagenicity Test. Mutat Res 1975;31:347-364.

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5. Operation of the Technic Model 301 Reverse Osmosis Pre-Treatment Water System and the Corning Model MP-1 Glass Still. LAIR Standard Operating Procedure OP-STX-94, Presidio of San Francisco, California: Letterman Army Institute of Research, 29 July 1985.

6. Ames Salmonella/Mammalian Microsome Mutagenesis Test. LAIR Standard Operating Procedure OP-STX-1, Presidio of San Francisco, California: Letterman Army Institute of Research, 29 August 1986.

7. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 1956;218:97-106.

8. Brusick D. Genetic toxicology. In: Hayes AW, ed. Principles and methods of toxicology. New York: Raven Press, 1982:223-272.

APPENDIX A

CHEMICAL DATA

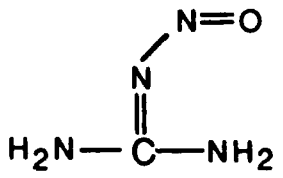
Chemical Name: Nitrosoguanidine

Chemical Abstracts Service Registry No.: 674-81-7

Lot Number: WCC-2-002

LAIR Code: TP 48

Chemical Structure:



Molecular Formula: CH4N4O

Molecular Weight: 88

Physical State: yellow powder

Analytical Data:

Nitrosoguanidine was analyzed by HPLC using conditions similar to those employed by Burrows et al.*1* Conditions were as follows: column, Brownlee RP-18 (4.6 mm x 25 cm); mobile phase, water; flow-rate, 0.8 ml/min. The effluent was monitored at 255 nm. The retention times for nitrosoguanidine and nitroguanidine were 4.4 and 6 min, respectively. The HPLC data demonstrated that the nitrosoguanidine contained approximately 2.5% nitroguanidine.*2* IR (KBr) 3378, 3096, 1690, 1649, 1508, 1341, 1266, 1134, 1088, 1035, 690, 668 cm-1.*3*

Solubility:

A saturated solution of nitrosoguanidine in water was prepared at room temperature. A 1:500 dilution of this solution produced an absorbance of 0.533 units. Using an extinction coefficient of 13,305 L/moles.cm, the concentration of nitrosoguanidine in the original saturated solution was calculated to be 1.763 mg/ml.*4*

Stability:

Stable for at least 4 hours in HEPES buffer (pH 7.3) at 37°C.*5*

Source: Alan Rosencrance
US Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Maryland

1Burrows EP, Brueggeman EE, Hoke SH. Chromatographic trace analysis of guanidine, substituted guanidines and s-triazines in water. Chromatog 1984;16:494-8.
2Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 37. Letterman Army Institute of Research, Presidio of San Francisco, CA.
3*Ibid.* p 30.
4Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-01-006, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.
5Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 46. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX B-1

RAW DATA TABLE

REVERTANTS/PLATE

NITROSOGUANIDINE (TP 48)

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
WITHOUT S-9								
NEG CONTROL	0.0 mg	101	14	105	178	16	11	21
(START RUN)		79	12	107	193	8	14	24
		61	10	97	174	11	13	26
NEG CONTROL	0.0 mg	100	12	115	158	12	8	13
(END RUN)		101	16	126	159	10	6	10
		85	19	117	178	8	8	15
WITH S-9								
NEG CONTROL	0.0 mg	104	45	134	283	13	10	26
(START RUN)		92	36	116	275	9	18	29
		100	46	99	280	13	6	27
NEG CONTROL	0.0 mg	140	36	149	288	12	15	28
(END RUN)		113	29	*	*	10	12	22
		113	17	*	*	10	11	27

*plate contaminated

APPENDIX B-2
RAW DATA TABLE
POSITIVE CONTROLS: REVERTANTS/PLATE

COMPOUND*	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
2-AA	2.0 µg		377	565			376	566
			285	476			266	806
			232	649			313	344
2-AF	2.0 µg	730	1683	682				1235
		501	1641	634				1299
		486	1726	802				971
BP	2.0 µg	244	118	335			46	43
		259	133	405			55	62
		251	125	357			44	118
MITO C	0.5 µg				1341			
					1339			
					1301			
MNNG	2.0 µg			1601				
				1304				
				1475				
MNNG	20.0 µg					1801		
						2340		
						2530		
NQNO	2.0 µg	289						
		147						
		320						

*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

APPENDIX B-3
RAW DATA TABLE
REVERTANTS/PLATE
NITROSOGUANIDINE (TP 48)

WITHOUT S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP 48	1.05 mg	70	25	107	171	21	15	15
		101	17	108	176	20	10	16
		76	20	*	158	14	9	14
TP 48	0.8 mg	94	19	114	170	19	9	13
		94	14	117	171	14	11	14
		83	26	99	151	13	13	15
TP 48	0.6 mg	91	17	76	157	17	26	15
		90	13	84	182	15	15	17
		93	10	69	178	10	7	9
TP 48	0.4 mg	75	25	115	180	13	9	20
		76	14	91	183	14	17	13
		83	15	111	155	18	4	15
TP 48	0.2 mg	79	18	105	118	14	9	15
		91	22	104	158	23	13	14
		93	16	97	127	19	9	11
TP 48	0.1 mg	65	15	101	174	17	6	12
		97	9	97	142	12	7	14
		87	17	111	180	15	8	15

*plate contaminated

APPENDIX B-4
RAW DATA TABLE
REVERTANTS/PLATE
NITROSOGUANIDINE (TP 48)

WITH S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP 48	1.05 mg	135	32	103	217	16	7	25
		119	37	*	238	8	18	29
		107	30	*	254	9	9	31
TP 48	0.8 mg	113	35	126	275	26	8	33
		99	30	121	256	11	8	19
		112	40	131	243	10	10	28
TP 48	0.6 mg	106	21	104	237	17	15	29
		118	37	105	245	16	18	20
		114	32	*	237	20	11	21
TP 48	0.4 mg	128	15	100	266	11	12	21
		147	34	*	279	6	9	20
		139	39	*	247	12	16	20
TP 48	0.2 mg	108	23	119	245	20	9	22
		120	20	108	202	17	10	19
		122	21	119	236	12	11	27
TP 48	0.1 mg	114	30	134	205	9	6	11
		122	28	*	255	11	4	25
		80	30	*	243	*	11	28

*plate contaminated

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